DEPARTMENT OF NOTES, REVIEWS, ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In the absence of these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

A RELIABLE METHOD FOR OBTAINING AMŒBA FOR CLASS USE

By Charles A. Kofoid

It is a common experience of the teacher in elementary biology to find that his supply of *Amœba* for class use is uncertain in source, variable in amount, and not infrequently fails at the appointed time, or is so scanty that pupil and teacher both find more experience in searching for the elusive creatures than in profitable observation. Those who have available an *Oscillaria* rich margin at some outlet of sewer or of a drain where a fairly permanent greenish-black felt of this alga is present, can often secure an abundant supply of large *Amœba proteus*. Decaying *Ceratophyllum* from pond or lake waters also yields this *Amœba* in abundance for a brief period in the process of decomposition in the laboratory aquarium. A permanent *Amœba* culture of this species might be arranged and regularly kept up in a culture house with supply of spring water suitably enriched. A culture of this sort of an apparently as yet undescribed *Amœba* was maintained at Lincoln, Nebraska, by Miss Powers and supplied widely to the laboratories of the country from Atlantic to Pacific for several years, but was unfortunately destroyed by purification treatment of the city water supply.

In our laboratories at the University of California we have for several years applied the principle of pure mixed cultures in a crude way to the production of amœbas in quantity for class use with such success that we offer it as one solution of the problem of supply for large classes where the elimination of loss of time in searching for the animals on the slide is desirable. The amœba

we have cultivated in a small soil amœba, *Nægleria gruberi* (Schardinger) originally described by Schardinger (1899) from the human intestine in a case of diarrhea. It is improbable, in view of the use of pure culture methods in his work, that his culture was contaminated from dust or water; though it is not impossible. The amœba in reality may have been recovered from the fæces after transit in an encysted condition through the intestine of the patient having been introduced in food or water. This is apparently the same amœba which Wherry (1913) later isolated from the water supply of Oakland, California.

There are certain disadvantages in its use. It is very small, about 9 to 30 microns, is not very mobile at room temperatures, lacks prominent pseudopodia in most cases, and perhaps worst of all, enflagellates on slight provocation, often at a very inopportune time, and quite without regard to class schedules. The compensating advantages are (1) the great numbers easily obtained, ten or a dozen in a single high-power field, and thus the entire elimination of the necessity of search for the animals; (2) the occurrence of cysts, amœboid, and flagellate stages and of both exogenous and endogenous budding; (3) ease and certainty (in our experience) of securing the cultures; and (4) ease with which slides of the various stages may be prepared. A full account of its life-history, as far as known, is now in press in the "University of California Publications in Zoology" by Dr. Charlie W. Wilson (1915) now of Mills College, Oakland, California.

The methods of culture which we employ for laboratory supply are as follows: We use as culture dishes enameled instrument trays 6 by 10 inches in size which fit readily in the horizontal autoclave, and accomodate each over fifty 22mm. floating cover glasses. As nutrient culture medium we use the filtered fluid from a mixture of 50 grams each of lettuce leaves, horse manure, soda cracker, and garden soil boiled for half an hour in a liter of creek water or untreated tap water. This fluid is sterilized for 30 minutes in the autoclave and when thoroughly cool is shaken up with a small quantity of soil and the water poured in the previously sterilized pan to the depth of 2 centimeters and covered with a glass plate. The pans should not be placed in the direct sunlight.

Clean cover glasses are floated carefully by dropping them on the surface of the culture fluid and are best removed by bent cover glass forceps. Soil bacteria and the soil amœbas appear within a few hours, and multiply rapidly, and are at a maximum in about four days after the culture is started, the period varying according to a number of factors such as temperature, richness of the medium, and certain unknown chemical conditions. The medium should not be too rich for best results and may be diluted to one-half or less the strength above indicated by use of a larger proportion of water. The numbers of amœbas are less in the poorer cultures, but there is danger of excessive bacterial growth and fermentation if the medium is too rich.

The amœbas rise to the surface and adhere in both amœboid and encysted stages to the under surface of the cover glass in astounding numbers. For class use it is only necessary to make certain of infected soil by a trial culture, and of the time interval between seeding the culture and the period of maximum abundance of amœbas under the conditions of temperature, stock of culture medium in use, etc. It is desirable to use a uniform, or the same stock of culture medium for both the preliminary test and final culture. When these factors are determined it is safe to proceed and to rely upon the culture to yield the amœbas as planned. It is possible to plant new cover glasses in the culture after removing the first supply and within 24 hours to have a new lot available. The cultures run down rapidly at first and then slowly for weeks or months after their brief maximum.

We have found it advisable to keep a stock in culture and to seed from it by adding a cubic centimeter of old culture fluid to the unseeded new medium instead of using the original soil material. Such a culture has now been continued in our laboratory for over thirty months.

The conditions which stimulate enflagellation are apparently access of oxygen and new food supply. For this reason cover glasses should be used promptly on removal. They not infrequently yield enflagellating amœbas within an hour and usually the whole culture on the cover glass follows suit. Encystment is very common and occurs both with abundant and with scanty food supply. Stained

preparations are readily made of the amœboid and encysted stages by floating the cover glass with its attached amœbas on warm Schaudinn's fluid amœba side downwards for a few moments, and then staining by the customary procedure in Heidenhain's iron hæmatoxylin. Amœboid, budding and encysted stages are abundant in such preparations and enflagellating, exflagellating stages and the flagellate stage itself are all to be found by careful search but are obviously more difficult to obtain in this way since they do not adhere habitually to the substrate.

Many of the amœbas are in stages of cell division, and many cysts are binucleate simulating sexual phases. Care is essential in interpreting these stages as sexual phenomena, which are as yet not proven to occur in this amœba. Chromidial extrusion simulating maturation phenomena also occurs in the cysts and many puzzling nuclear conditions, probably degenerative or involution phenomena, will be found in stained preparations from cultures, especially in the period of the decline from the maximum numbers.

Cultures easily become contaminated and ciliates from the soil are usually inoculated with the amœbas. For this reason it is wise, if one wishes to keep a permanent culture going, to isolate a ciliate-free cover glass for seeding the permanent culture.

Our experience leads us to believe this culture method adds a valuable resource to our biological laboratory and increases our means of control of an abundant supply of this very important type essential both for instruction and research.

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